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(54) Title: METHODS AND MATERIALS FOR OPTIMIZATION OF ELECTRONIC HYBRIDIZATION REACTIONS

(57) Abstract

The following inventions relate to discoveries concerning the various parameters, electrolytes (buffers), and other conditions which improve or optimize the speed of DNA transport, the efficiency of DNA hybridization reactions, and the overall hybridization specificity in microelectronic chips and devices. In particular, this invention relates to the discovery that low conductance zwitterionic buffer solutions, especially those containing the amino acid Histidine prepared at concentrations of ~50 mM and at or near the pI (isoelectric point ~pH 7.47), provide optimal conditions for both rapid electrophoretic DNA transport and efficient hybridization reactions. Hybridization efficiencies of at least a factor of 10 relative to the next best known buffer, Cysteine, are achieved. Test data demonstrate an approximately 50,000 fold increase in hybridization efficiency compared to Cysteine.

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DESCRIPTION

Methods And Materials For Optimization Of Electronic Hybridization Reactions

Field of the Invention

This invention relates to buffers and electrolytes for use in electronic devices adapted for medical diagnostic, biological and other uses. More particularly, it relates to buffers and electrolytes for advantageous use with DNA hybridization analysis carried out on microelectronic medical diagnostic devices.

Background of the Invention

Recently, there has been increasing interest in devices which combine microelectronics and molecular biology. One such system is disclosed in "ACTIVE PROGRAMMABLE ELECTRONIC DEVICES FOR MOLECULAR BIOLOGICAL ANALYSIS AND DIAGNOSTICS", Serial No. 08/146,504, filed November 1, 1993, now issued as United States Patent No. 5,605,662, incorporated herein by reference. The systems disclosed therein will be referred to as APEX systems. APEX systems are able to perform a wide variety of functions which are advantageously used in molecular biology reactions, such as nucleic acid hybridizations, antibody/antigen reactions, clinical diagnostics, and biopolymer synthesis.

APEX-type devices utilize buffers and electrolytes for their operation. A buffer has been defined as a chemical solution which is resistant to change in pH on the addition of acid or alkali. See., e.g., Dictionary of Biotechnology, Second Edition, James Coombs, Stockton Press. As stated there, "traditionally, buffers based on inorganic salts (phosphate, carbonate) and organic acid salts (acetate, citrate, succinate, glycine, maleate, barbiturates, etc.) were used in biological experiments."

It is the object of this invention to discover buffers and electrolytes which are advantageously used in molecular biology electronic devices which perform hybridizations, reactions, diagnostics or synthesis.

Summary of the Invention

The following inventions relate to our discoveries concerning the various parameters, electrolytes (buffers), and other conditions which improve or optimize the speed of DNA transport, the efficiency of DNA hybridization reactions, and the overall hybridization specificity in our APEX microelectronic chips and devices. In particular, this invention relates to the discovery that low conductance zwitterionic buffer solutions, especially those containing the amino acid Histidine prepared at concentrations of 10-100 mM, preferably about 50 mM, and at or near the pI (isoelectric point ~pH 7.47), provide optimal conditions for both rapid DNA transport and efficient hybridization reactions. Hybridization efficiencies of at least a factor of 10 relative to the next best known buffer, Cysteine, are achieved. Test data demonstrate an approximately 50,000 fold increase in hybridization efficiency compared to Cysteine.

Brief Description of the Drawings

Fig. 1 is a plan view of a checkerboard arrangement utilizing a histidine buffer.

Detailed Description of the Invention

There are various physical parameters which relate to the electrophoretic transport of DNA and other charged analytes in various types of electrolyte/buffer solutions. Certain of the devices, e.g., Applicant's APEX device as described in United States Patent No. 5,605,662, referenced above, are basically DC (direct current) electrical devices which generate electric fields on the surface of the device. These fields, in turn, cause the

electrophoretic transport of charged molecules to occur between oppositely (+/-) biased microlocations on the device surface. By contrast the so-called Genosensor (impedance sensors), see, e.g., Hollis et al, "Optical and Electrical Methods and Apparatus for Molecular Detection", WO93/22678, and dielectrophoresis devices, see, e.g., Washizu 25 Journal of Electrostatics, 109-123, 1990, involve the use of AC electric fields. An important distinction related to these devices is that when these AC fields are applied, there is essentially no net current flow in any of these systems, i.e, there is no electrophoretic propulsion for transport of the charged molecules. APEX type devices produce significant net direct current (DC) flow when a voltage is applied, which is recognized as "the signature of electrophoresis". In electrophoresis, the migration of ions or charged particles is produced by electrical forces along the direction of the electric field gradient, and the relationship of current and voltage are important to this technology. The electrophoretic migration shows itself macroscopically as the conduction of electric current in a solution under the influence of an applied voltage and follows Ohm's law:

$V=RxI$

V is the electric potential

R is the electric resistance of the electrolyte [$VxA^{-1}=R(\Omega)$]

I is the electric current [A].

The resistance of the solution is the reciprocal of the conductance which can be measured by a conductometer. The conductance depends mainly on the ionic species of the buffer/electrolytes and their concentration; therefore these parameters are very important for electric field related molecular biology technology. The basic current/voltage relationships are essentially the same for the APEX technology as for any other electrophoretic system, although the electric fields produced are in truly microscopic environments.

There are unique features of the APEX system regarding the various ways of sourcing the current and voltage, and how the current and voltage scenarios have been found to improve the performance of such systems. In particular, various DC pulsing procedures (linear and logarithmic gradients) appear to provide improved hybridization stringency.

Electrophoretic Transport Versus Ionic Strength

It is well established in the field of electrophoresis that there is a logarithmic decrease in the mobility of the charged analyte species (proteins, DNA, etc.), which is inversely proportional to the square root of the ionic strength of the electrolyte solution (see page 83 and Fig. 3.16 in "Capillary Electrophoresis: Principles and Practice", R. Kuhn and S. Hoffstetter, Springer-Verlag, 1993). At any given constant electric field strength, as the electrolyte concentration decreases relative to the analyte species (protein, DNA, etc.), the analyte will be transported at a faster rate. Similar results demonstrating this effect for a danyslated amino acid have been shown by J.J. Issaq et. al., Chromatographia Vol. 32, #3/4, August 1991, pages 155 to 161 (see in particular Fig. 3 on page 157). Results demonstrating this effect for DNA is different electrolyte solutions has been shown in P.D. Ross and R.L. Scruggs, Biopolymers Vol. 2, pages 231 to 236, 1964 (see in particular Fig. 1, page 232).

Ionic Strength/Conductance Relationship

For those non-buffering electrolytes (sodium chloride, potassium chloride, etc.) which involve completely dissociated anion and cation species in solution (Na^+ <---> Cl^-, K^+ <---> Cl^-, etc.), the ionic strength and conductance are equivalent, i.e., the conductance will usually be proportional to the ionic strength. For those buffering electrolytes (phosphate,

acetate, citrate, succinate, etc.) which are in their dissociated states (example: 2 Na^+ <---> PO_4^{--2}), the ionic strength and conductance will usually be equivalent, i.e., conductance is proportional to the ionic strength. For those buffering electrolytes [Good Buffers (MOPS, HEPES, TAPS, Tricine, Bicine), Amino Acid Buffers, Ampholytes, etc.] which can have a zwitterionic species (*no net charge at their pI*), the conductance will decrease by approximately a factor of 10 for every pH unit difference between the isoelectric point (pI) and the (pKa). For example, an amino acid in its zwitterionic state ($^-$OOC-CH(R)-NH_3^+) will have a conductance value which will be approximately 1000 fold lower than when the "*amino acid moiety*" has a full net positive charge (HOOC-CH(R)-NH_2^+ <---> X^-), or a full negative charge (Y^+ <--->$^-$ OOC-CH(R)-NH_2). Thus, a formal negative or positive charge develops on the amino acid moiety as it moves away from its pI, and the conductivity and ionic strength will begin to correlate. However, when at or near the pI the conductance will be much lower than is expected for that given ionic strength or concentration. When used at or near their pI's, electrophoresis texts refer to the Good Buffers and amino acid buffers as having "low conductances at high ionic strength or concentration" (see page 88 of Capillary Electrophoresis: Principles and Practice", R. Kuhn and S. Hoffstetter, Springer - Verlag, 1993). A commonly used electrophoresis buffer "Tris-Borate" actually has a significantly lower conductivity than would be expected from its ionic strength or concentration. This may be due to the "tris cation" and "borate anion" forming a relatively stable zwitterionic complex in solution. The conductivity of a 100 mM Tris-Borate solution was determined to be 694 μS/cm, which is approximately 20 times lower than would be expected from its ionic strength, and is roughly equivalent to a 5 mM sodium phosphate or sodium chloride solution. Table 1 shows conductivity measurements of a number of transport buffers.

Solution/Buffer	Measurement 1	Measurement 2	Measurement 3	Average/Std. Deviation
10 mM $MgCl_2$	1.95 mS/cm	2.02 mS/cm	2.13 mS/cm	2.03+/-0.09 mS/cm
1 mM $MgCl_2$	174 µS/cm	208 µS/cm	177 µS/cm	186+/-18.8 µS/cm
0.1 mM $MgCl_2$	16.9 µS/cm	16.7 µS/cm	18.3 µS/cm	17.3+/-0.87 µS/cm
10 mM NaCl	1.07 mS/cm	1.10 mS/cm	1.18 mS/cm	1.12+/-0.057 mS/cm
1 mM NaCl	112 µS/cm	115 µS/cm	111 µS/cm	112.7+/-2.08 µS/cm
0.1 mM NaCl	8.80 µS/cm	8.98 µS/cm	10.5 µS/cm	9.43+/-0.93 µS/cm
20 mM $NaPO_4$	2.90 mS/cm	2.79 mS/cm	3.00 mS/cm	2.90+/-0.11 mS/cm
10 mM $NaPO_4$	1.40 mS/cm	1.44 mS/cm	1.48 mS/cm	1.44+/-0.04 mS/cm
1 mM $NaPO_4$	122 µS/cm	128 µS/cm	136 µS/cm	128.7+/-7.0 µS/cm
50 mM TRIS	3.50 mS/cm	3.14 mS/cm	3.40 mS/cm	3.35+/-0.19 mS/cm
10 mM TRIS	572 µS/cm	562 µS/cm	583 µS/cm	572+/-10.5 µS/cm
250 mM HEPES	141 µS/cm	144 µS/cm	158 µS/cm	147.6+/-9.07 µS/cm
25 mM HEPES	9.16 µS/cm	9.44 µS/cm	10.5 µS/cm	9.7+/-0.71 µS/cm
3.3 mM NaCitrate	964 µS/cm	964 µS/cm	1.03 mS/cm	986+/-38.1 µS/cm
5 mM NaSuccinate	1.05 mS/cm	960 µS/cm	1.01 mS/cm	1.01+/-0.045 mS/cm
5 mM NaOxalate	1.02 mS/cm	1.03 mS/cm	1.12 mS/cm	1.06+/-0.055 mS/cm
10 mM NaAcetate	901 µS/cm	917 µS/cm	983 µS/cm	934+/-43.5 µS/cm
250 mM Cysteine	27.4 µS/cm	17.3 µS/cm	23.5 µS/cm	22.7+/-5.09 µS/cm
Milli-Q water	<0.5 µS/cm			Detection limit of
				0.1 cell too low

Table 1

Zwitterionic Buffers/Conductance/Transport Rate

Certain advantages exist regarding the rate or speed of electrophoretic transport of DNA when using Zwitterionic buffers (Good buffers, amino acid buffers), or the Tris-Borate buffer at or near their pIs. These are: 1) such buffers can be used at relatively high concentrations to increase buffering capacity, 2) their conductances are significantly lower than other types of buffers at the same concentration, and 3) one gains the advantage of higher electrophoretic transport rates for the analyte of interest (DNA).

Zwitterionic Buffer Capacity at the Isoelectric Point (pI)

Amino acid buffers do have buffer properties at their pI's. While a given amino acid may or may not have its "highest buffering capacity" at its pI, it will have some degree of buffering capacity. Buffer capacity decreases by a factor of 10 for every pH unit difference between the pI and the pKa; those amino acids with three ionizable groups (histidine, cysteine, lysine, glutamic acid, aspartic acid, etc.) generally have higher buffering capacities at their pI's than those amino acids with only two dissociations (glycine, alanine, leucine, etc.). For example, histidine pI = 7.47, lysine pI=9.74, and glutamic acid pI=3.22, all have relatively good buffering capacity at their pIs, relative to alanine or glycine which have relatively low buffering capacities at their pIs (see A.L. Lehninger, Biochemistry, 2ed, Worth Publishers, New York, 1975; in particular Fig. 4-8 on page 79, and Fig. 4-9 on page 80). Histidine has been proposed as a buffer for use in gel electrophoresis, see, e.g., U.S. Patent 4,936,963, but hybridization is not performed in such systems. Cysteine is in a more intermediate position, with regard to buffering capacity. The pI of cysteine is 5.02, the pKa for the α carboxyl group is 1.71, the pKa for the sulfhydryl is 8.33, and the pKa for α amino group is 10.78. An acid /base titration curve of 250 mM cysteine,

shows that cysteine has a better "buffering capacity" at ~ pH 5 than a 20 mM sodium phosphate. In the pH 4 to 6 range, the buffering capacity of cysteine is significantly better than 20 mM sodium phosphate, particularly at the higher pH. However, in these pH ranges the conductance of the 250 mM cysteine solution is very low ~23 μS/cm, compared to 20 mM sodium phosphate which has a value of ~2.9 mS/cm, a factor of 100 times greater. Figure 1 shows the Conductivity Measurements of Various Transport Buffers.

Several electrophoretic techniques developed over 20 years ago are based on the ability to separate proteins in zwitterionic buffers "at their pIs." These techniques are called Isoelectrophoresis, Isotachophoresis, and Electrofocusing (see chapters 3 and 4 in "Gel Electrophoresis of Proteins: A Practical Approach" Edited by B.D. Hames & D. Rickwood, IRL Press 1981). Various amino acid buffers and Good buffers were used for these applications, all at their pI's (see Table 2, page 168 of the above reference).

DNA Transport in Low Ionic Strength and Low Conductance Buffers

A series of fluorescent checkerboard experiments were carried out using 2.5% agarose coated 5580 chips and the ByTr-RCA5 fluorescent probe. We were able to achieve rapid (6 second) checkerboard addressing in all of the following systems: (1) 250 mM HEPES (low conductance), (2) 10 μM sodium succinate, (3) 10 μM sodium citrate, and (4) distilled water. The results for sodium citrate are shown in Figure 1. While, some types of low conductance or low ionic strength solutions may have somewhat better characteristics, checkerboard addressing and rapid DNA transport (6 to 12 second DNA accumulation on an 80 μm pad) were achieved using all of these systems. Additionally, DNA addressing APEX chips in distilled water is possible because the DNA (itself a polyanion) is the

electrolyte present in the bulk solution which provides the conductance. Fig. 1 shows a plan view of an APEX chip using histidine.

Relationship of Electrophoretic Transport Rate and the Cation/Anion Species

In addition to the fact that the mobility of the charged analyte species (DNA, proteins, etc.) is related to the ionic strength of the electrolyte solution, the mobility is also greatly influenced by the nature of the cation and anion species in the electrolyte solution (see pp 89 of "Capillary Electrophoresis: Principles and Practice" reference). This particular point is demonstrated for DNA transport in the above Biopolymers, Vol. 2, pp. 231-236, 1964 reference. Figure 1 on page 232 of this reference shows the change in DNA mobility when using electrolytes with different univalent anions ($Li^+ > Na^+ > K^+ > TMA^+$) at the same ionic strength. Basically, different cations can have different association constants with the DNA phosphate groups, and/or change the hydration spheres around the DNA molecules, which leads to a change in their transport rate.

The instant invention relates to our discoveries concerning the various parameters, electrolytes (buffers), and other conditions which improve or optimize the speed of DNA transport, the efficiency of DNA hybridization reactions, and the overall hybridization specificity in electric field molecular biology devices, especially APEX microelectronic chips and devices. In particular, this invention relates to our discovery that low conductance zwitterionic buffer solutions containing the amino acid Histidine prepared at concentrations of 10-100 mM, especially about 50 mM, at or near the pI (isoelectric point ~7.47), provide optimal conditions for both rapid electrophoretic DNA transport and efficient hybridization reactions. This advantage of the Histidine buffer is particularly important for the APEX chip type devices.

These particular devices (as opposed to the micromachined type devices) have limitations as to the amount of current and voltages that can be applied. This limitation makes it difficult to achieve both rapid transport and efficient hybridization using the same buffer system. In these cases, DNA transport was carried out in a low conductance buffer (Cysteine or Alanine) where the limited current/voltage still produced rapid transport. Under these conditions the DNA accumulated at the test site, but did not hybridize as efficiently. After transport in these low conductance buffers, the solution was changed to a high salt buffer (> 100 mM sodium chloride or sodium phosphate) which then produced an efficient hybridization at the test site.

Table 2 shows the results for a series of experiments which correlate the parameters of buffer capacity, pH, and the conductivity, with DNA accumulation and hybridization sensitivity (efficiency) using the APEX chip device.

Solution	Buffer Capacity pH 4-10		pH at PI	Conductivity (μS)	Relative DNA Transport Rate	SA-Biotin T12 Sensitivity	Hybridization Sensitivity of DNA
β-Alanine	pK_1 - 3.6 pK_2 - 10.2	+	7.3	10.0	+++++ (fastest)	3×10^6	
Taurine	pK_1 - 1.5 pK_2 - 8.7	+/-	4.6	4.5	++++	$> 7.5 \times 10^{10}$	
Cysteine	pK_1 - 1.7 pK_2 - 8.3 pK_3 - 10.8	+/-	5.2	25.0	++++	3×10^7	7.5×10^{10}
Histidine	pK_1 - 1.8 pK_2 - 6.0 pK_3 - 9.0	+++	7.6	212.0 (172.0 hi purity)	+++	3×10^6	3×10^6
Lysine	pK_1 - 2.2 pK_2 - 8.9 pK_3 - 10.3	++	9.6	477.0	++	$> 7.5 \times 10^{10}$	
$NaPO_4$	Complex	+	7.4[1/]	1,400.0	+ (slowest)		

TABLE 2

In particular, Table 2 shows the effect of various zwitterionic amino acid buffers [β-Alanine, Taurine, Cysteine, Histidine, Lysine, and Sodium Phosphate (not a zwitterionic buffer)] on the hybridizability of the transported target DNA to the specific capture DNA at the test site. As to transport, the conductivity generally correlates with transport under the same field conditions. β-Alanine, Taurine and Cysteine show excellent transport, Histidine shows good transport, and Lysine and $NaPO_4$ show fair transport. The DNA hybridization sensitivity is reported for "normal DNA" which has negatively charged polyanionic phosphate backbone. In addition to the hybridization sensitivities, Table 2 also reports the sensitivity for the streptavidin/biotin DNA probe capture affinity.

Table 2 clearly shows the correlation of DNA transport (accumulation) with low conductivity (β-Alanine, Taurine, Cysteine, Histidine). The table shows good

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1/ 20mM $NaPO_4$ adjusted to pH 7.4.

sensitivity for the streptavidin/biotin probe affinity reaction using β-Alanine, Cysteine, and Histidine. As reflected in the sensitivity data in Table 2, Histidine provides over four orders of magnitude better hybridization efficiency then either Cysteine or other buffers, such as 20 mM $NaPO_4$. The improvement relative to Cysteine is at least a factor of 10, more especially a factor of 10^2, and most especially at least a factor of 10^4. Most importantly Table 2 shows that the DNA hybridization sensitivity (efficiency) is very good for the Histidine buffer. Thus of all the zwitterionic amino acid buffers presently tested, Histidine is the only one which provides both good transport and good DNA/DNA hybridization efficiency.

It is believed that the low conductivity of the Histidine buffer system accounts for the rapid DNA transport (accumulation). There are several possible explanations as to why the Histidine buffer produces relatively efficient DNA/DNA hybridization. One advantage may be the good buffering capacity of Histidine. With its pI at 7.47, Histidine will buffer well under both acidic or basic conditions (see A.L. Lehninger, Biochemistry, 2ed, Worth Publishers, New York, 1975, Fig. 4-9 on page 80). The APEX chip produces acid at the positive electrode where the DNA is accumulated for hybridization, and Histidine may effectively buffer these conditions. More importantly, under these acidic conditions (pH<5) the protonation of the imidazole group on the Histidine begins to convert the molecule into a dicationic species. It may be the case that this dicationic species with a positively charged α-amino group and a positively charge imidazole group may help to promote hybridization and stabilize the DNA/DNA hybrids formed at the positive electrode on the APEX chip. Cations, dications, and polycations are known to help stabilize DNA/DNA hybrids by reducing the repulsion of the negatively charged phosphate backbones on the double-stranded DNA structure. It is also possible

that the DNA/DNA/Histidine may also form some type of stabilizing adduct from other electrochemical products being produced at the positive electrode (hydrogen peroxide, etc.)

While the instant embodiment utilizes naturally occurring Histidine, this invention is fully applicable to other natural or synthetic compounds which have good buffering capacity, low conductivity (or zwitterionic characteristics) and have properties which allow DNA hybridization to be stabilized by charge stabilization or adduct formation.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

Claims

1. A method for transporting and hybridizating target nucleic acids in a microelectronic device having at least one test site bearing a capture nucleic acid, comprising the steps of:

(1) applying a low conductance buffer to the device,

(2) applying current to the device to produce an electric field at the test site,

(3) transporting the target nucleic acids to the test site, and

(4) hybridizing the target nucleic acids to the capture nucleic acid at the test site with a hybridization efficiency which is at least a factor of 10 times greater than for Cysteine under the same conditions.

2. The method of claim 1 wherein the low conductivity buffer is a zwitterionic buffer.

3. The method of claim 2 wherein the zwitterionic buffer includes histidine.

4. The method of claim 3 wherein the histidine was prepared at a concentration of about 10-100 mM.

5. The method of claim 3 wherein the histidine was prepared at or about the isoelectric point.

6. The method of claim 1 wherein the isoelectric point is about pH 7.47.

7. The method of claim 1 wherein the buffer entity stabilizes hybridization between the target nucleic acids and the capture nucleic acid.

8. The method of claim 7 wherein the buffer entity is a natural compound with low conductivity.

9. The method of claim 7 wherein the buffer entity is a natural, zwitterionic compound.

10. The method of claim 7 wherein the buffer entity is a synthetic compound with low conductivity.

11. The method of claim 7 wherein the buffer entity is a synthetic, zwitterionic compound.

12. The method of claim 1 wherein the hybridization efficiency is at least a factor of 100 times greater than for Cysteine under the same conditions.

13. The method of claim 1 wherein the hybridization efficiency is at least a factor of 1,000 times greater than for Cysteine under the same conditions.

14. The method of claim 1 wherein the hybridization efficiency is at least a factor of approximately 50,000 times greater than for Cysteine under the same conditions.

15. The method of claim 1 wherein the buffer entity reduces repulsion between the capture nucleic acid and the target nucleic acids.

16. The method of claim 1 wherein the buffer reduces adduct formation between the capture nucleic acid and the target nucleic acids.

17. A method for enhancing the electrophoretic transport and hybridization efficiency of target nucleic acids in a microelectronic hybridization device including a microlocation test site having a capture nucleic acid, comprising the steps of:

applying a low conductivity buffer to the device,

applying power to the device to cause electrophoretic transport and accumulation of the target nucleic acids at a microlocation test site on the device, and

hybridizing the target nucleic acids with an efficiency which is at least a factor of 10 times greater than for Cysteine under the same conditions.

18. The method of claim 17 wherein the low conductivity buffer is a zwitterionic buffer.

19. The method of claim 18 wherein the zwitterionic buffer includes histidine.

20. The method of claim 19 wherein the histidine was prepared at a concentration of about 10-100 mM.

21. The method of claim 19 wherein the histidine was prepared at or about the isoelectric point.

22. The method of claim 17 wherein the isoelectric point is about pH 7.47.

23. The method of claim 17 wherein the buffer entity stabilizes hybridization between the target nucleic acids and the capture nucleic acid.

24. The method of claim 23 wherein the buffer entity is a natural compound with low conductivity.

25. The method of claim 23 wherein the buffer entity is a natural, zwitterionic compound.

26. The method of claim 23 wherein the buffer entity is a synthetic compound with low conductivity.

27. The method of claim 23 the buffer entity is a synthetic, zwitterionic compound.

28. The method of claim 17 wherein the hybridization efficiency is at least a factor of 100 times greater than for Cysteine under the same conditions.

29. The method of claim 17 wherein the hybridization efficiency is at least a factor of 1,000 times greater than for Cysteine under the same conditions.

30. The method of claim 17 wherein the hybridization efficiency is at least a factor of approximately 50,000 times greater than for Cysteine under the same conditions.

31. The method of claim 17 wherein the buffer entity reduces repulsion between the capture nucleic acid and the target nucleic acids.

32. The method of claim 17 wherein the buffer reduces adduct formation between the capture nucleic acid and the target nucleic acids.--

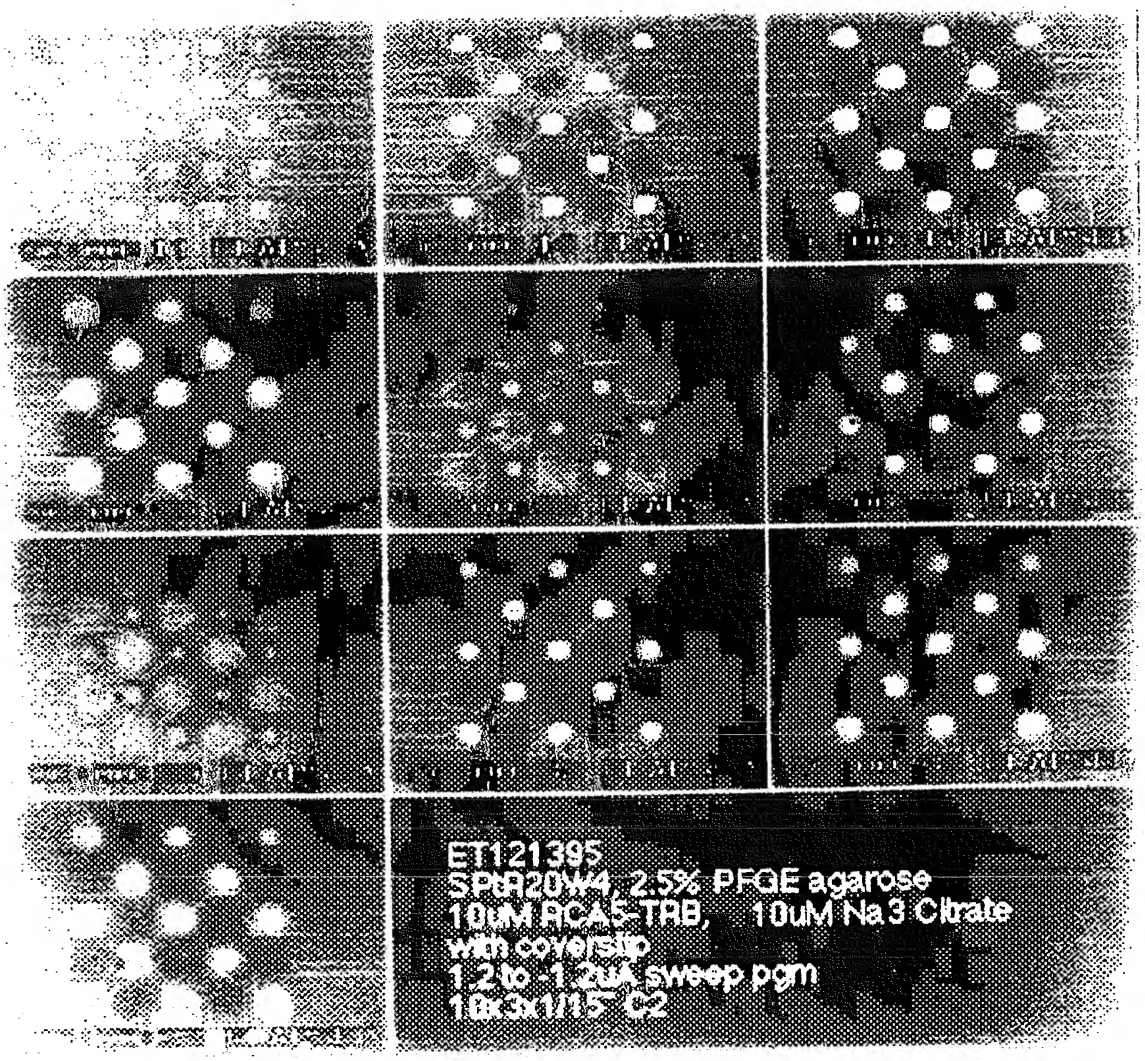

FIG. 1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14489

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL : 204/450, 468; 435/6
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 204/450,468;435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS, USPAT, JPOABS, WPIDS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 4,936,963 A (MANDECKI ET AL) 26 June 1990 (26/06/90) see entire document.	1-11 --- 1-11
Y	US 5,436,129 A (STAPLETON) 25 July 1995 (25/07/95) see entire document.	1-11
E	US 5,593,838 A (ZANZUCCHI ET AL) 14 January 1997 (19/01/97) see entire document.	1-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 25 SEPTEMBER 1997	Date of mailing of the international search report 0 5 DEC 1997
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